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Assessment of the antimicrobial and antioxidant activity of seaweeds and seaweed based foods

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ABSTRACT

Seaweeds have been used for preparation of low caloric foods such as salads, soups. Jelly, Jam and puddings for many centuries are in raw or semi processes of form. They are known as source of secondary metabolites which can be used of as antioxidants and antimicrobials. Present study was carried out to evaluate the antimicrobial and antioxidant activities of methanol extract of different seaweeds namely *Ulvalactuca*, *Sargussumwightii*, *Gracilaria edulis*, *Gracilaria verrucosa*, *Kaphaphycusalevarezii* and their extracted polysaccharides agar agar, carrageenan, alginic acid and seaweed based soup and jam. Seaweeds were collected from Southern and North west coast of Sri Lanka. The different concentrations from 0.2-0.5 mg/ml methanol extract of seaweed and seaweed based products were screened against human pathogens such as *Staphylococcus aureus*, *Salmonella*, *Escherichia coli.* Crude extracts of all seaweed samples were tested for their antimicrobial activity using disc diffusion method. The antioxidant activity was determined by DPPH free radical scavenger method and total phenol content was measured by the Folin-ciacalteau's procedure. Antimicrobial activities with inhibition diameters in all methanol extracts were ranged from 1.0 to 8.0mm. Extracts of *Kapphaphycusalverazii* exhibited maximum antibacterial activity against all the bacteria tested. The minimum inhibition diameters 1.23 mm and 1.1 mm were observed for *U. lactuca* against both *Escherichia coli* and *Salmonella respectively.* The seaweed polysaccharide and seaweed based products showed lower effect in inhibition of the pathogenic bacteria when compared to raw seaweeds. *Sargassumwightti* exhibited significantly high phenol content at 2.67 GAE/g of seaweed extract and also had highest DPPH scavenging activity

(p<0.05) with a 50% inhibition (EC 50) level. The methanol extracts of *Gracilaria edulis* had second highest 1.24 GAE/g total phenol content (p<0.05) and DPPH scavenging activity. The results suggested *Kapphaphycus* has effective inhibition activity against pathogens while *Sargassumwightti* can be utilized as efficient source of natural antioxidant to be used in food and pharmaceutical industry.

Key words: antimicrobial activity, *antioxidant*, polysaccharides, *seaweeds*, metabolites

1. INTRODUCTION

Seaweeds are important components of marine ecosystem having various biological activities (Bouhlal *et al.,* 2011; Kayalvizhi *et al.,* 2012). Seaweeds are macroscopic algae with rich in bioactive compounds and they found attached to the bottom of relatively shallow coastal waters. They grow in the intertidal, shallow and deep sea areas up to 180 meter depth and also in estuaries and back waters on the solid substrates such as rocks, dead corals, pebbles, shells and other plant materials. They grouped under three divisions namely, Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae) depending on their nutrient and chemical composition.

The genus *Ulva* has a great potential as commercial product because of its fruitful taste and varied chemical composition and quality (Selvin et al., 2004). *Kappaphycus alvarezzi*, a red alga (Division Rhodophyta) is used for various nutritional products including antioxidant for use as heath food or neutraceutical supplement. *Kappaphycus alvarezzi* has nutritive and antioxidant property. Different parts of the thalli are also known to differ in their antimicrobial potential (Fayaz *et al.,* 2005). Some microbes are sociable but most of them are very pathogenic, *Escherichia coli, Staphylococcus aurous* and salmonella are causes diseases like food borne gastroenteritis, urinary tract infections and upper respiratory complications (Jawetz *et al*., 1985; Leven, 1987). Antibiotics are used to control the pathogenic organism, but microbes are becoming more resistant common antibiotics which has necessitated the development of new alternatives (Smith *et al*., 1994). Need for screening of the new antibacterial agents from available sources natural and seaweed are one of the present trends. (Subba Rangaiah *et al*., 2010). Traditionally, seaweeds have been used in the treatment of various infectious diseases. Screening of antimicrobial compounds from seaweeds is vital and increasing demand for therapeutic drugs (Prasad *et al*., 2010). The present study was aimed to investigate the antioxidant and antibacterial activity of *Sargussumwightii*, *Gracilaria edulis Kappaphycusalvarezii* and *Ulvalactuca* seaweeds and seaweed based polysaccharides, foods such as soup, *Ulva* Jam, against three human pathogenic organisms both gram positive and gram negative bacteria.

2. METHODOLOGY

Collection of samples

The seaweed species *Gracilaria verricos*a, *Gracilariaedulis, Kappuphycusalvarezii* (red seaweed), *Sargussum* species (brown seaweed), *Ulvalactuca*and*Ulvareticulata* (green seaweed) were collected from North western and South west coastal belt of Sri Lanka respectively and transported to laboratory in 0^0C by keeping in an insulated box. The seaweed samples were thoroughly washed with seawater and then freshwater to remove epiphytes and other dirt particles. Dried, grinded seaweed were stored in the -18^0C. The samples were used to determine the phenolic content, total flavonoid as well as for antioxidant activity.

Preparation of samples

The pulverized moisture free seaweed sample (20g) was extracted using200ml of methanol by continuous homogenizing for 72 hours. The extraction was repeated many times to obtain a required quantity of extract. Consequently, the extract was concentrated in a rotary evaporator at 40^0C and divided in to two parts. The stock solution was prepared by dissolved in seaweed methanol extract powder 50,000µg in ml of methanol for determination of antioxidant activity. One part of dried powder was dissolved in distilled water containing less than 0.2% of methanol and stored at -20^0C until used for determination of antimicrobial activity.

Antioxidant activity

Assay was carried out with DPPH (2, 2 diphenyl-2-picryl hydrazyl hydrate) (Sigma, USA) using a spectrophotometric method (Brand Williams *et al.,* 1995). Freshly prepared DPPH solution was used for each experiment. Reaction mixture was prepared using 2.5 ml of 6.5 x 10^{-5}M DPPH solution and 0.5 ml (50,000µl/ml) of sample dissolved in methanol. The control sample was prepared by adding 0.5 ml of methanol to 2.5 ml of 6.5 x 10^{-5}M DPPH solution instead of the sample. Dilution series were prepared by taken 0.5 ml from reaction mixture and mixed with 0.5 ml methanol (1:1) solution triplicates in each. All samples were incubated at room temperature for 30 minutes in dark and absorbent measured at 540 nm using UV–160 Vis spectrophotometer (SHIMADZU UV mini 240). The

percentage of DPPH radical scavenging activity was determined using following equation mentioned. Butyl Hydroxy Toluene was used as the reference standard. The sample concentration vs inhibition percentage was plotted and 50% scavenging activity was estimated as IC_{50} value.

$$\% \text{ scavenging activity} = A_0 - A_s / A_0 \times 100$$

Where,

A_s - Absorbent of the DPPH solution of the control sample,

A_0- Absorbance of the DPPH solution in the presence of plant extract.

Total phenol content

The total phenol content of crude extract was determined in spectrophotometrically using Folin-Ciocalteu reagent (Slinkard *et al*., 1977). Point one gram (0.01g) of dried seaweed powder was mixed with 5.0 ml of hot 70% methanol in a water bath at 70°C for 10 minutes. Samples were cooled to the room temperature (30°C) and centrifuged at 3500 rpm and decanted the supernatant and diluted using 70% methanol. The reaction mixture was prepared using 0.5 ml of extracted sample with 2.5 ml of Folin-Ciocalteu reagent which diluted 10 times using 70% methanol. Sodium carbonate 7.5% (w/v) solution was added after three minutes to the above mixture and kept at room temperature for two hour incubation period. Absorbance was measured at 765 nm using the UV-Visible spectrophotometer (SHIMADZU UV-160) expressed as Gallic acid equivalent /g (GAE/g) using the equation obtained from the calibration curve for Gallic acid.

Total flavonoid content:

The total flavonoid content of each extract was determined using colorimetric meter according to (Chang, *et al*. 2002) standards as mg Rutin equivalent /g. 0.5ml of sample solution (50000µg/ml) was mixed with 2ml of distilled water and subsequently with 0.15 ml 5% of $NaNo_2$ solution, After 6 minute's incubation, and 0.15 ml of 20g/l aluminium trichloride ($AlCl_3$) in methanol solution was added and allowed to stand for 6 min, followed by adding 2 ml of 4% NaOH solution to the mixture. The mixture was made up to 25ml with methanol and mixed well. The absorbance at 510nm was read in UV-Visible spectrophotometer (SHIMADZU- UV-160) after incubation for 15 min.

Antibacterial activity

Bacterial strains

The Gram positive bacteria used *Staphylococcus aureus, gram negative bacteria Salmonella typhimuriumand Escherichia coli*were obtained from the microbiology unit of Institute of Post-harvest Technology/National Aquatic Resource Research and Development Agency. All cultures were kept on nutrient agar slants and stored at -4^{0}C, except the initial stock cultures which were stored at -80^{0}C.

Preparation of algal disc for antibacterial activity

Agar cultures of the test microorganisms were prepared as described by Mackeen et al, (2000). Three to five similar colonies were selected and transferred to 5 ml nutrient broth with a loop and the broth cultures were incubated for 24 h at 37ºC. For screening sterile (hot air at 16^{0}C for one hour) 6mm diameter filter paper disc were impregnated (20µl) range from 80mg/ml to 0.125mg/ml concentrations. A bacterial suspension 50µlin (Macfarland scale about 1.5 x 10^8) was spread on Nutrient agar (pH – 7.4).The discs contain seaweed extract at ten different concentrations (from 80 mg/ml to 0.125mg/ml) were placed on surface on the NA agar plates. Plates were incubated at 37^{0}C for 24hours. The antimicrobial activity was quantified as the diameter (mm) of the growth inhibition zones. Clear inhibition zones around the disc indicated the presence of antimicrobial activity.

Minimum inhibitory concentration (MIC)

Each *seaweed* extracts against bacterial isolates was tested in nutrient agar plates by pour plate method according to the JSC, (1981). Seaweed extracts were dissolved in 0.2% methanol to obtain 128mg/ml stock solutions and diluted to get a concentration of 1.25, 2.50, 5, 10, 20, 40 and 80 128 mg/ml. 50µl of standardized suspension of the test organism were inoculated into seaweeds extracts incorporated nutrient agar plates. The culture plates were incubated at 37^0 C for 24hours. The lowest concentration which did not show any growth of tested pathogenic bacteria was determined as Minimum inhibitory concentration.

Statistical analysis

Statistical analysis was conducted using SPSS 22 (2015) version software package on the data of triplicates of each parameter. The comparison of mean values of each treatment were done using one way analysis of variance (ANOVA) followed by turkey test.

3. RESULTS AND DISCUSSION

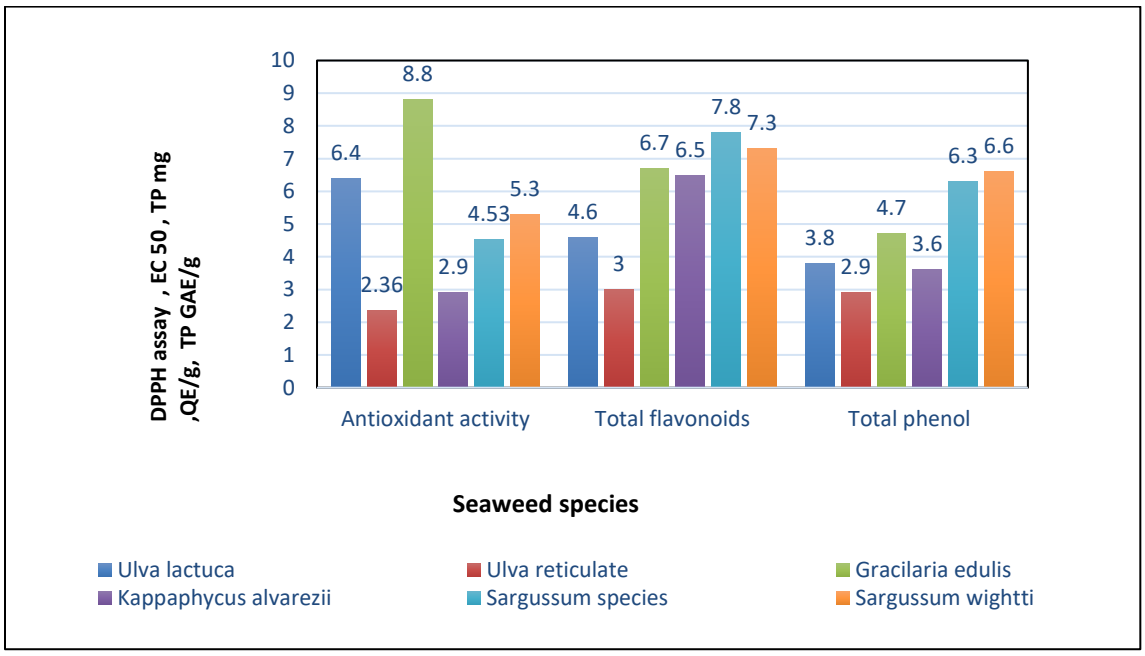


Figure 1 Mean value of antioxidant activity EC 50 Total flavonoids mg QE/g, Total phenol mg GAE/g in different seaweed species

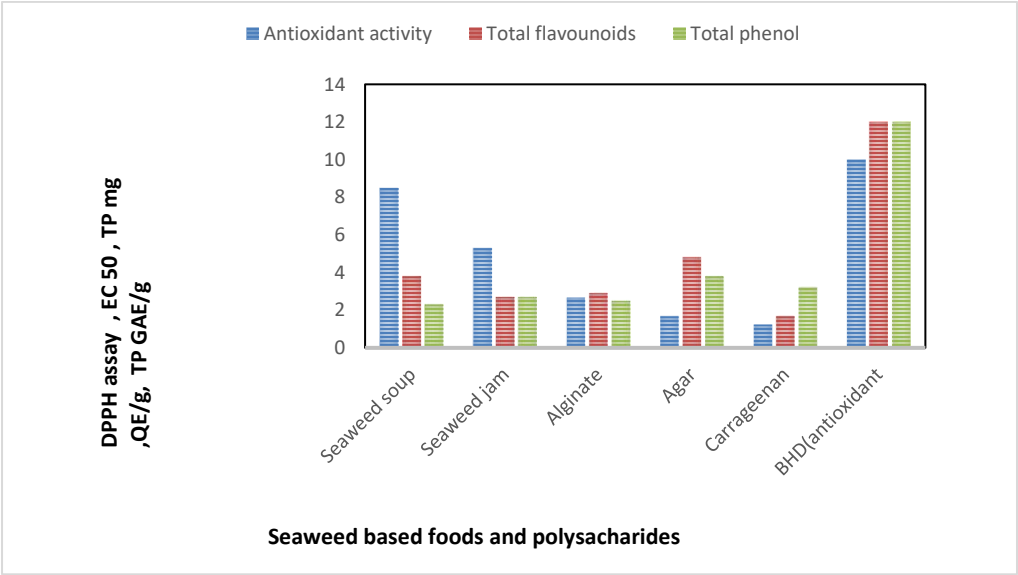


Figure 2 Mean value of antioxidant activity EC $_{50}$ Total flavonoids mg QE/g, Total phenol mg GAE/g in different seaweed species

DPPH radical scavenging activity of methanol seaweed extracts

The antioxidant activities of all six seaweeds were significantly different (p <0.05), and EC_{50} levels ranged from 2.36 to 8.8 µg/ml. In *Sargussum wightii* showed highest activity achieving 50% inhibition of DPPH radical at a concentration of 4.5. In red seaweeds also

achieved good inhibition at EC_{50} levels of range from 2.9 and 5.5 µg/ml of extract (*Kapphaphycus* and *Gracilaria verrucosa* respectively). In green seaweed inhibition range was observed from 2.53-3.3µg/ml. *Ulva reticulat* and *U. lactuca* had observed significantly lower antioxidant activity with at 2.5 and 50 µg/ml of EC_{50}.Wang *et al.* (2009) and Yan *et al.* (1999), who confirm the results of present study also found that brown algae contained higher amounts of polyphenols and DPPH radical scavenging activity than red and green algae. However, Chandini *et, al.* (2008) reported low levels of DPPH radical scavenging activity in brown seaweeds, in the range of 17.79 to 23.16% at concentration of 1000 µg/ml seaweed extract. Also, Wang*et al.* (2009) reported on the antioxidant activities of Icelandic seaweeds indicating that brown species exhibited the most effective scavenging ability than others. In extracted polysaccharides (agar, carrageenan and alginates) were observed radical scavenging activity lower than the raw seaweeds. The alginate recorded lower DPPH radical scavenging activity while agar was recorded inhibitory activity 3.6µg/ml. In order to the seaweed based products the DPPH scavenging activity was highest in seaweed based soup whereas lowest value indicated seaweed based jam respectively 5.3µg/ml to 8.9 and µg/ml.

Total flavonoids and Phenolic content

The total flavonoid content and Phenolic content of the dried seaweed extracts and seaweed based products are given in Table 2. Total flavonoids in the seaweeds ranged from 2.7 to 6.6 mg QE/g of extract. Brown seaweed species, *Sargussum wightii* contained significantly higher total flavonoid contents (p <0.05) than the other seaweeds studied. Green seaweeds, *Ulva lactuca* and *U. reticulata* had the lowest total flavonoid contents at 2.9 and 3.8 mg QE/g extract, respectively. (p < 0.05). Kahkonen *et al.*(1999) stated that flavonoids are probably the most important natural phenolic due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and potential therapeutic agents against a wide variety of diseases (Ross and Kasum, 2002). Flavonoids of the studied seaweeds based products ranged from 2.1 to 3.7 mg QE/g of extract. The content of flavonoids from the seaweed based products wasn't significantly higher than the other species (p < 0.05). The carrageenan. Alginates and agar contained lower total flavonoids than the brown seaweeds and was in the range 1.67 to 4.8 QE mg/g of seaweed extract.

Table 2 Zone of inhibition of methanol extract of seaweed and seaweed products against selected food pathogenic bacteria

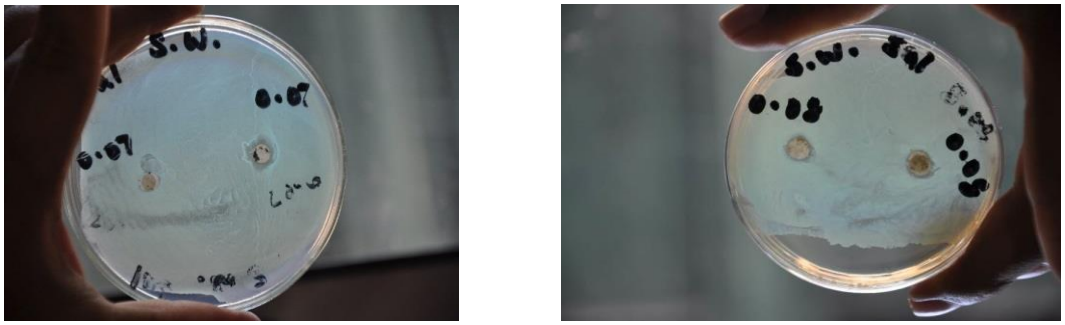
Concentration of methanol extract (mg/ml)	*Sargussum wightii*	*Ulvalalactuca*	*Kapphaphycus alverazii*	Agar	Carrageenan	Vegetable soup with seaweed	*Ulva* Jam
	Zone of inhibition (mm) for *Staphylococcus aureus*						
8	5±0.02	5±0.6	9.16±0.01	3±0.1	3±0.2	5±0.23	5±0.34
4	4.3±0.01	4.6±0.02	5±0.02	3±0.03	3±0.14	5±0.12	4±0.04
2	3.2±0	4.1±0.01	5±0.001	3±0.01	2±0.05	4±0	2±0.03
1	2.5±0.1	4.1±0.1	3.2±0.23	2.1±0.01	1.1±0.1	4.2±0.23	3.5±0.1
0.50	1±0.2	3±0.24	2±0.03	1±0.02	1±0.34	3.3±0.56	2.67±0.12
0.250	1±0.3	3±0.03	2±0.02	1±0.1	1±0.56	3±0.12	2±0.3
0.125	1±0.13	2±0.02	0±0.2	1±0.03	0±0	2±0.32	1±0.03

Table 3 Zone of inhibition of methanol extract of seaweed and seaweed products against selected food pathogenic bacteria *Salmonella typhi*

Concentration of methanol extract (mg/ml)	*Sargussum wightii*	*Ulvalactuca*	*Kapphaphycu salverazii*	Agar	Carrageenan	Soup	*Ulva* jam
	Zone of inhibition (mm) for *Salmonella typhimurium*						
8	4±0.023	5±0.04	7±0.02	4±0.02	4±0.01	6±0.01	6±0.1
4	3±0.01	5±0.34	7±0.031	4±0.02	4±0.00	5±0.23	5±0.2

2	3±0.04	3±0.2	6±0.01	3±0.001	3±0.02	5±0.2	5±0.3
1	2±0.03	2±0.0	6±0.04	3±0.01	2±0.00	4±0.01	5±0.4
0.5	1±0.025	2±0.0	5±0.03	2±0.03	1±0.1	3±0.01	4±0.56
0.25	1±0.021	1±0.01	2±0.05	0±0.02	1±0.1	3±0.00	2±0.1
0.125	1±0.03	1±0.01	1±0.0	0±0.0	0±0	1±0.02	1±0.2

Food processes food safety regulators and regulatory agencies are continuously concern over the high and growin number of disease outbreaks caused by some pathogenic and spoilage micro organisms. Consumers are demanding foods which are fresh natural and minimally processed without synthetic preservatives. Zone of inhibition of *Staphylococcus aureus* ranged from 0-9.16mm followed by *Salmonella* ranged from 0-7*cm E. coli range from 0-5cm Kapphaphycus alvarezii* extracts (Table 2, 3 & 4.) exhibited strong inhibition activity at the highest concentration (80 mg/ml) against *S. aurous,E. coli* and *Salmonella,* (9.16, 5, and 5.66mm respectively). This was significantly higher than all other concentrations of extracts tested ($p < 0.05$). However, even at the lowest concentration of extract tested (0.05 mg/ml) the activity against all tested bacteria was 6mm, which was stronger than that of the other seaweeds.



Plates 1 Antibacterial counts in different concentrations

This could possibly be due to the high concentration of polysaccharides in *Kapphaphycus* which are known to have antimicrobial properties (Fayazet, al., (2005) reported that *Kappaphycus alvarezii* has nutritive and antimicrobial property. Different parts of the thalli are also known to differ in their antimicrobial potential (Fayaz*et al.,* 2005). Brown seaweed *Sargussum wightii and Ulva lactuca extracts* showed similar range (1-5mm) of inhibition activity against *Staphylococus aureus*. The zone of inhibitory activity of gradually decreased respectively *Kaphaphycus, Ulva* jam, seaweed soup *Sargussum* and *Ulva* causing substantial growth inhibition 0.125 mg/ml concentration of agarand carrageenan had no antimicrobial activity against four strains of tested pathogenic bacteria. This would possibly be due to high concentration of phenolic and flavonoids compounds are removed by during processing of agar. Above 50mg/ml concentrations which are known to be good antimicrobial properties 2.5mg/ml concentrations were show moderate activity (3mm-5mm) inhibition zone all tested microorganisms. The red seaweed *Kapphaphycus*, vegetable soup with seaweed, seaweed jam and *G. edulis* respectively reveals that they can be used as potential antimicrobial agent against *S. aureus.*

Table 4 Zone of inhibition of methanol extract of seaweed and seaweed products against selected food pathogenic bacteria Escherichia coli

concentrations of methanol extract mg/ml	Seaweed and seaweed products						
	Sargussum wightii	*Ulvalactuca*	*Kapphaphycus Alvarezii*	Agar	Carrageenan	Soup	*Ulva*Jam
	Zone of inhabitation (mm) in *Escherichia coli*						
8	3±0.02	3.5±0.034	5.5±0.03	3±0.001	2±0.03	3±0.03	6±0.23
4	3±0.1	3.3±0.021	5.2±	3±0.002	2±0.01	3±0.023	6±0.35
2	3±0.03	2±0.02	4±0.01	2±0.03	2±0.02	2±0.01	5±0.56
1	2±0.02	1±0.1	4±0.3	2±0.02	1±0.023	2±0.01	5±0.021
0.5	2±0.03	1±0.02	2±0.05	1±0.001	1±0.01	2±0.02	2±0.032
0.250	2±0.03	1±0.012	2±0.042	1±0.3	1±0.012	1±0.02	1±0.01

0.125	1±0.2	0±0.03	0±0.031	0±0	0±0	0±0	1±0.02

Minimum Inhibitory Concentration (MIC) was significantly higher in *Ulva lactuca* against *Stapphylococus aureus* followed by *Sargussum wightii* 0.9 mg/ml MIC concentration against E. coli. *Kapphaphycus* showed significantly lower MIC value than other seaweeds. In the MIC assay, S aurous and *E.coli* were most sensitive for *Kapphaphycus alvareizii* extract at lower concentrations as compared to other pathogens. The results of the present study reveal that Gram positive bacteria were more susceptible to methanolic extract seaweed than Gram negative bacteria. From the above preliminary studies on the antibacterial activities, all the organic extracts of seaweeds showed promising activity against all the test pathogens. The results indicate significant capacity and future scope for the use of seaweed against a wide range of microbial populations.

Table 5 Minimum inhibitory concentrations (MIC) of methanol extracted seaweed, against for suspension of pathogenic microorganism

	Bacterial strains		
MIC (seaweed extract)	*Salmonella typhimurium* mg/l	*Staphylococcus aureus* mg/l	*Escherichia coli* mg/l
Sargussum wightii	0.74±0.02	0.75±0.01	0.9±01
Ulva *lactuca*	0.73±0.02	0.93±0.01	0.7±0.01
Gracilaria *verrucosa*	0.78±0.2	0.56±0.2	0.54±0.1
Kapphaphycus alvarezii	0.8±0	0.89±0	0.59±0

4. CONCLUSION

The results of the present study indicated that Sri Lankan seaweeds and seaweed based products *Kapphaphycus alvarezii, Sargussum wightti, Ulva lactuca, Ulva reticulate, Gracilaria edulis Gracilaria verrucosa , agar, carrageenan, and alginic acid , soup and Ulva jam* adequately displayed antioxidant and antimicrobial activities respectively in decreasing order. Total phenolis, condensed tannins and flavonoids in the red *kapphaphycus* and brown seaweeds were highest and it had the highest properties of antimicrobial and antioxidant content. *Staphylococcus aureus* is highly susceptible gram positive bacteria to *kapphaphycus alvarezii* than for other species. The results of the present study indicated that the antimicrobial activity of seaweed extracts were concentration dependent and antimicrobial properties are strong in higher concentration. Six different laboratory developed seaweed based food product also indicate successful antioxidant activity. Seaweed mixed vegetable soup has available phenolic contents than some raw seaweed. This is a promising finding, as there may be a potential to utilize such extracts depend on seaweed species in food products.

These bio active compounds in seaweeds are excellent free radical scavengers and potent natural phenolic antioxidants and antibiotics for commercial exploration. These compounds are removed during process in to seaweed based foods and results in the less contribution to antimicrobial and antioxidant activity. It appears that seaweeds available from our coasts possess significant antibiotic capabilities and thus deserve a place in marine biotechnology programs to evaluate the bio active products.

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